Subterranean Biology 9: 103-112, 2011
doi: 10.3897/subtbiol.9.2513

Assessing copepod (Crustacea: Copepoda) species richness at different spatial scales in northwestern Romanian caves

Ioana N. MELEG [1,*], Frank FIERS [2], Oana T. MOLDOVAN [1]

[1] "Emil Racoviţă" Institute of Speleology, Clinicilor 5, PO BOX 58, 400006 Cluj-Napoca, Romania; * e-mail: ioana.meleg@hasdeu.ubbcluj.ro
[2] Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000, Bruxelles, Belgium
* corresponding author

ABSTRACT
The aim of the present study was to assess copepod species richness in groundwater habitats from the Pădurea Craiului Mountains, Transylvania (northwestern Romania). Five species richness estimators (one asymptotic, based on species accumulation curves, and four non-parametric) were compared by testing their performances in estimating copepod species richness at three hierarchical spatial scales: cave, hydrographic basin, and karstic massif. Both epigean and hypogean species were taken in account. Two data sets were used in computing copepod species richness: 1. samples collected continuously during one year (dripping water) and seven months (pools) from five caves, and 2. samples collected from pools in twelve additional caves (data gathered from literature). Differences in copepod species richness among caves and hydrographic basins suggest that local environmental features are important in determining local species richness trends.

Key words: copepods, species richness, groundwater habitats, Romania

INTRODUCTION

The vadose zone, i.e. the unsaturated karstic layer between surface and groundwater table, consists of the epikarst (the uppermost unsaturated zone of the carbonate bedrock) and the void network through which water percolates towards the phreatic zone (Mangin 1994; Ford and Williams 2007). Communities inhabiting the vadose zone are diverse, consisting of surface dwellers and specialized subterranean species (Rouch 1968; Lescher-Moutoué 1973; Gibert 2001; Pipan 2005; Pipan and Culver 2007). The faunal assemblages of the vadose zone can be studied by sampling drip water systems and pools (Pipan 2005).

Assessing species diversity is a challenge for ecologists, due to difficulties in collecting and identifying all species and estimating their relative abundance with a limited sampling effort (Chao et al 2005). This challenge becomes even more difficult for diversified communities with many rare representatives (Colwell and Coddington 1994; Chazdon et al 1998; Colwell et al 2004; Magurran 2004), such as the case of the vadose zone with diverse and confined aquatic assemblages.

In the vadose zone of the caves from the Pădurea Craiului Mountains (western Romanian Carpathians) the most often encountered taxa in decreasing number are: harpacticoid and cyclopoid copepods, insect larvae (mainly Diptera Chironomidae), Collembola, Ostracoda, Oligochaeta, Nematoda, Amphipoda, Acari, Gastropoda and Isopoda (Moldovan et al 2007; Meleg et al 2011). Among these taxa, copepods are the most diversified and abundant, probably the most characteristic taxon dwelling the voids of the vadose zone, as was also mentioned in other karst systems of Southern Europe (Stoch 1997, 2000; Brancelj 2002; Pipan and Brancelj 2001, 2004a, b; Pipan 2005; Sket et al 2004; Camacho et al 2006).

Species richness is the simplest parameter used in assessing community diversity (Chao 2005), being the most frequently used indicator (Gaston 1996) in biodiversity conservation and ecological research (Brown et al 2001). Therefore, species richness as the core component of biodiversity (Gaston 1996) may be used in assessing the groundwater diversity of the Pădurea Craiului Mountains, partly still unknown. Among the methods developed for estimating species richness, species accumulation curves and non-parametric estimators are widely used (Bunge and Fitzpatrick 1993; Colwell and Coddington 1994; Chao 2005).

In the present study we assessed copepod species richness by using different species richness estimators for copepod communities in the vadose zone of the Pădurea Craiului Mountains at different spatial hierarchical scales (cave, hydrographic basin, and massif), and along a vertical gradient in two different habitats (void networks and pools). The obtained results are used to define the best species richness estimator for each spatial scale mentioned above.

Received 17 September 2010; Accepted 18 April 2011

MATERIALS AND METHODS

Study area

The Pădurea Craiului Mountains are a karstic "island" that lies in North-Western Romania. As part of the Apuseni Mountains (the western part of the Romanian Carpathians), they cover about 1150 km^2, with an altitude range between 300 and 1000 m a.s.l (Rusu 1988). In Pădurea Craiului Mountains a Natura 2000 Site of Community Interest (SCI) has been designated: Defileul Crişului Repede-Pădurea Craiului, code ROSCI0062. The Natura 2000 habitat type "caves not open to the public" represents the second habitat as surface in the SCI mentioned above (18.9%). All caves reported in the present study are located in this site.

Data analysis

Two data sets were included in the study. The first data set is represented by five caves from two hydrographic basins: the Crişul Repede basin (CR) with Ungurului Cave (UC), Vadu Crişului Cave (VC) and Peştera cu Apă din Valea Leşului Cave (LC), and the Crişul Negru basin (CN) with Ciur Izbuc Cave (CC) and Doboş Cave (DC) (Fig. 1). In these caves, the dripping water was sampled monthly over one year and the associated pools were sampled monthly over seven months. This set was used in computing species richness at cave and hydrographic basin scale and in different habitats (voids and pools). The second data set is represented by fauna from pools from 12 additional caves existing in the data base of the "Emil Racoviţă" Institute of Speleology (in Cluj). This set was used in computing species richness at the massif level.

The dripping water fauna was sampled according to the funnel method described by Brancelj (2004). Animals from the dripping water system and also those sampled directly from the associated pools were retrieved from the sampling device and pools respectively by a 100 µm mesh-sized hand net and fixed in 96% ethanol.

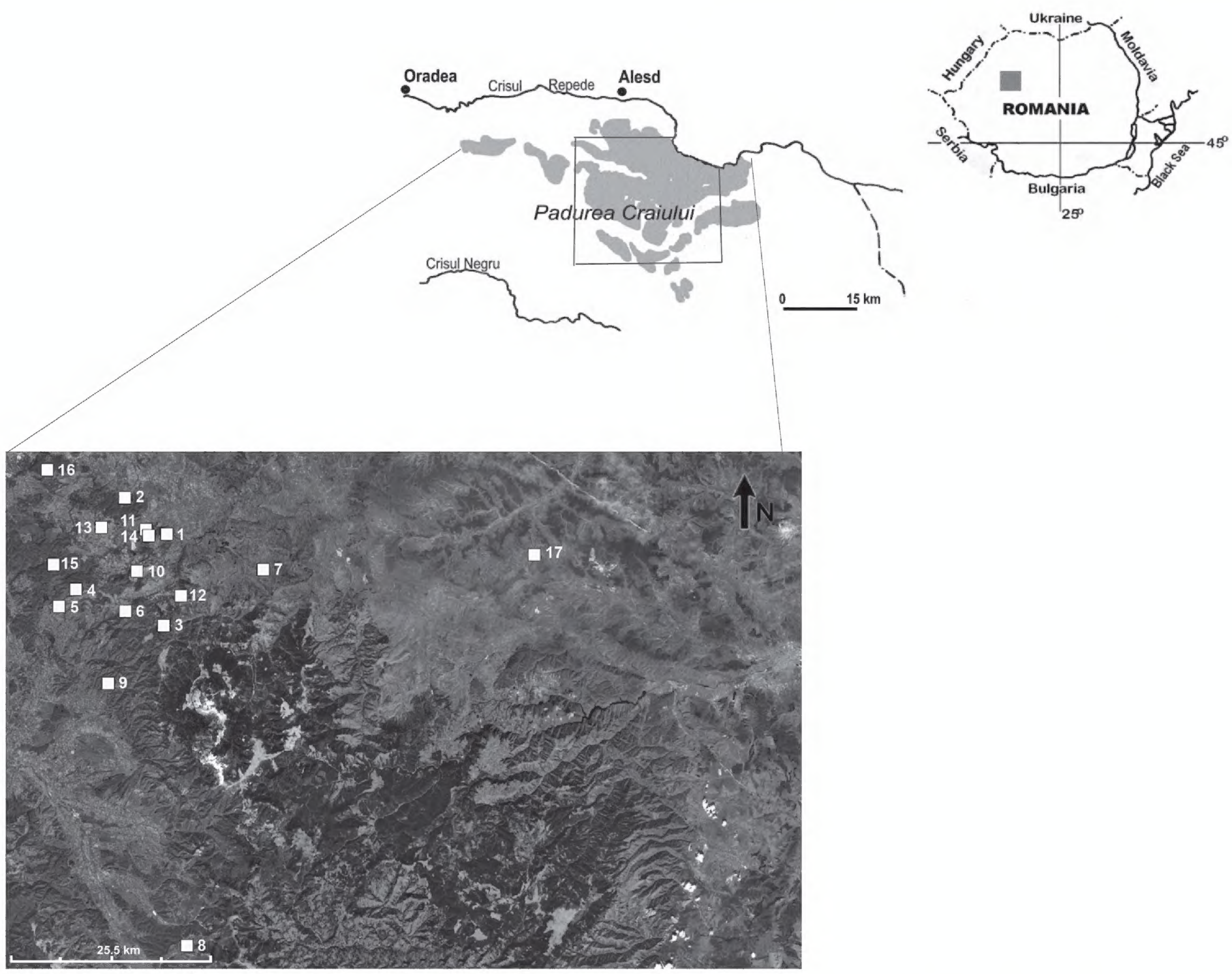


Figure 1 - Location of investigated caves in the Pădurea Craiului Mountains (north-western Romania): 1. UC; 2. VC; 3. LC; 4. CC; 5. DC; 6. Întorsuri Cave; 7. Săncuta Cave; 8. Cubleş Cave; 9. Meziad Cave; 10. Moanei Cave; 11. Vântului Cave; 13. Fanului Cave; Bătrânului Cave; 14. Napiştileu Cave; 15. Vizu Cave; 16. Igriţa cave; Gălăşeni Cave.

We considered two ecological categories, epigean *vs.* hypogean species (Rouch 1968; Rouch 1982; Rouch and Carlier 1985), based on their occurrence in the subterranean environment and in the surface habitats respectively.

Four non-parametric species richness estimators were selected. For the first data set, Chao 1 and Abundance-based Coverage Estimator (ACE) as abundance-based estimators were used, while incidence-based estimators (Chao 2 and Incidence-based Coverage Estimator (ICE) were used for the second data set, due to the lack of abundance data (Chao et al 1993; Lee and Chao 1994; Chazdon et al 1998). Chao estimators were used for the vadose community due to the presence of many endemic species encountered and rare species which carried most of the information about the number of missing species, i.e. singletons (species known from a single individual) and doubletons (species known from two individuals) for Chao 1, uniques (species that occur in only one sample) and duplicates (species that occur in only two samples) for Chao 2. ACE and ICE were selected to overcome the species richness overestimation, in caves where the number of samples was low, and where very abundant species were found together with very rare species. Mao Tau procedure (expected species accumulation curves-SACs) was used to represent inventory exhaustivity. Comparisons of species richness estimators' performance on sample-based data were represented graphically, by plotting the estimated and observed species richness against the number of sampled sites (Chazdon et al 1998).

At the cave scale and at the basin scale, the monthly copepod abundance in each sample was included. Copepod incidence per cave was used in computing and plotting species richness at massif scale. The effect of non-random spatial distribution on the estimator performance was tested by modifying the degree of patchiness on samples at basin scale. The same pattern was examined also for hypogean/epigean species. This was performed in EstimateS software by defining a patchiness parameter ranging from 0 (random distribution) to 0.5 (moderate patchiness) and 0.9 (high degree of patchiness).

All computations were based on 50 randomized runs and were performed using EstimateS version 8.2. (Colwell 2009).

RESULTS

Thirteen copepod species were sampled from dripping water and pools in the five caves studied over one year (UC, VC, LC, CC and DC). Twenty-two copepod species collected from pools of 12 caves were added to the analysed data set (Table 1). The number of observed species and samples collected differed at all scales taken into account (cave, hydrographic basin, massif, drips and pools) (Table 2).

The SACs for the three caves from CR basin (UC, VC and LC) showed that sampling completeness was not achieved. When sampling is close to be exhaustive, the number of singletons should be close to zero which is not the case here, reinforcing the results obtained from SACs (Fig. 2 UC, VC and LC; Table 2). For UC and VC the number of species approached an asymptote, but still increased slowly. For UC and VC, Chao1 provided the least biased estimate of species richness, reaching a stable estimate of eight species in UC after 12 samples out of 13, and seven species in VC after 32 samples out of 33. For LC, both estimators failed to reach a stable value. The SACs for caves of the CN basin were asymptotic. The SAC obtained for CC, rapidly followed an asymptote, reaching 75 % of the maximum after eight samples and about 95 % after 18 samples out of 47. In DC the SAC asymptote was reached after eight samples out of nine (Fig. 2 CC, DC; Table 2). In CC, both Chao1 and ACE satisfied the criteria of ideal species richness estimators by performing well at low sample number: a stable estimate of five species was reached after only 29 samples out of 47. In DC, Chao1 was the best estimator with a stable value of three species, after seven samples out of nine. Both caves from CN basin had zero singletons and zero doubletons.

The data computed for CR and CN based on the samples collected in three and two caves respectively are plotted in Fig. 3 and summarized in Table 2. The species richness estimation at the CR and CN basin scale followed the species richness pattern estimated for caves belonging to the CR basin (UC, VC and LC) and to the CN basin (CC and DC) respectively. In CR, sampling completeness was not achieved, and the two non-parametric estimators failed to reach a stable value. In CN, the situation was different; the SAC did reach an asymptote, zero singletons were encountered and both Chao1 and ACE encountered the stable value of five species after 34 samples out of 52.

The effect of patchiness on estimated species richness was tested at basin level. The simulations showed that for CR the rate of species accumulation with sampled area was higher when species were distributed randomly among samples. Moreover, more the degree of patchiness increased (from 0.5 to 0.9), more the initial rate of species richness decreased (Fig. 3). In CN, the species richness estimators were not sensitive to patchiness. Furthermore, the Chao1 and ACE performed well at 0.9 and 0.5 degree of patchiness respectively, being less sensitive to sample size (Fig. 3).

At massif level, 20 species were found in six caves and 75% of the maximum number of observed species was reached after collecting in 12 caves. Chao2 and ICE did not reach a steady value, though it appeared they were approaching a stable estimate: around 60 species after 16 sampled caves; the number of uniques was very high: 19 (Fig. 4; Table 2). These two estimators performed well at a moderate (0.5) and high (0.9) degree of patchiness.

Table 1 - List of copepod species found in caves from Pădurea Craiului Mountains. Hydrographic basins are indicated between brackets (CR-Crişul Repede basin; CN-Crişul Negru basin). Data gathered from literature is indicated in bold; *-hypogean species; underlined-endemic species; 0-absence; +-presence.

Copepod species/cave	Ungurului (CR)	Vadu Crişului (CR)	Peştera cu apă din Valea Leşului (CR)	Ciur Izbuc (CN)	Doboş (CN)	**Întorsuri (CN)**	**Săncuta (CR)**	**Cubleş (Cugliş) (CN)**	**Meziad (CN)**	**Moanei (CR)**	**Vântului (CR)**	**Fanului (CR)**	**Bătrânului (between basins)**	**Napiştileu (CR)**	**Vizu (CN)**	**Igrita (CR)**	**Gălăşeni (CR)**
***Attheyella (Attheyella) crassa* Sars, 1862**	0	+	0	0	0	0	0	0	+	0	0	0	0	0	0	+	0
***Attheyella (Attheyella) wierzejskii wierzejskii* Mrazek, 1893**	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
***Bryocamptus (Bryocamptus)* cfr. *baikalensis* Borutzky, 1931**	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
***Bryocamptus (Bryocamptus) minutus* Claus, 1863**	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
***Bryocamptus (Bryocamptus) vejdovskyi* Mrazek, 1893**	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Bryocamptus (Echinocamptus) dacicus* (Chappuis 1923) ***	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
***Bryocamptus (Echinocamptus) echinatus* Mrazek, 1893**	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
Bryocamptus (Rheocamptus) caucasiscus Borutzky, 1930	+	+	+	+	+	0	0	0	+	0	0	0	0	0	0	0	0
***Bryocamptus (Rheocamptus) spinulosus* Borutzky, 1931**	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
***Bryocamptus (Rheocamptus) tatrensis* Minkiewicz, 1916**	+	+	0	0	0	0	0	0	+	+	0	0	0	0	+	0	0
***Bryocamptus (Rheocamptus) typhlops* Mrazek, 1893**	+	+	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0
***Bryocamptus (Rheocamptus) unisaetosus* Kiefer 1930 ***	+	0	0	0	0	0	0	0	0	+	0	0	0	0	+	0	0
***Bryocamptus (Rheocamptus) zschokkei zschokkei* Schmeil, 1893**	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bryocamptus sp. 1	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bryocamptus sp. 2	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bryocamptus sp. 3	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0
***Ceuthonectes serbicus* Chappuis 1924 ***	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
***Elaphoidella putealis* (Chappuis 1925) ***	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Maraenobiotus brucei carpathicus Chappuis, 1928	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Maraenobiotus vejdovskyi vejdovskyi Mrazek, 1893	0	+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
***Moraria (Moraria) brevipes* Sars, 1863**	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Moraria (Moraria) poppei* Mrazek, 1893**	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parastenocaris* sp. 1	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parastenocaris sp. 2*	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Parastenocaris sp. 3*	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
***Pesceus schmeili* Mrazek, 1893**	+	+	0	0	0	0	0	0	0	+	0	0	0	0	+	0	0

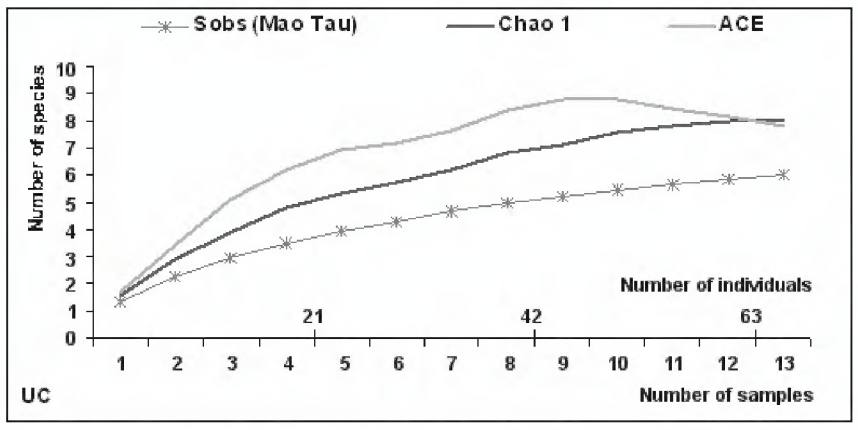
Copepod species/cave	Ungurului (CR)	Vadu Crişului (CR)	Peştera cu apă din Valea Leşului (CR)	Ciur Izbuc (CN)	Doboş (CN)	**Întorsuri (CN)**	**Săncuta (CR)**	**Cubleş (Cugliş) (CN)**	**Meziad (CN)**	**Moanei (CR)**	**Vântului (CR)**	**Fanului (CR)**	**Bătrânului (between basins)**	**Napiştileu (CR)**	**Vizu (CN)**	**Igriţa (CR)**	**Gălăşeni (CR)**
Spelaeocamptus spelaeus (Chappuis, 1925) *	+	0	+	+	+	+	0	+	+	0	0	0	+	+	0	+	+
***Acanthocyclops deminutus* (Chappuis 1925) ***	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
***Acanthocyclops kieferi* (Chappuis 1925) ***	0	+	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0
Acanthocyclops transylvanicus *	+	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0
***Diacyclops bisetosus* Rehberg, 1880**	0	+	0	0	+	0	0	0	0	0	+	0	0	0	0	0	0
Diacyclops stygius (Chappuis 1924) *	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
***Eucyclops serrulatus* Lilljeborg, 1901**	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Graeteriella unisetigera Graeter, 1908	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0
***Megacyclops viridis* Jurine, 1820**	0	+	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0
Paracyclops fimbriatus Fischer, 1853	+	+	0	0	0	0	+	0	0	+	0	0	0	0	0	0	0
Speocyclops troglodytes (Chappuis, 1923) *	0	+	0	0	0	+	0	+	0	0	+	0	0	0	0	0	0

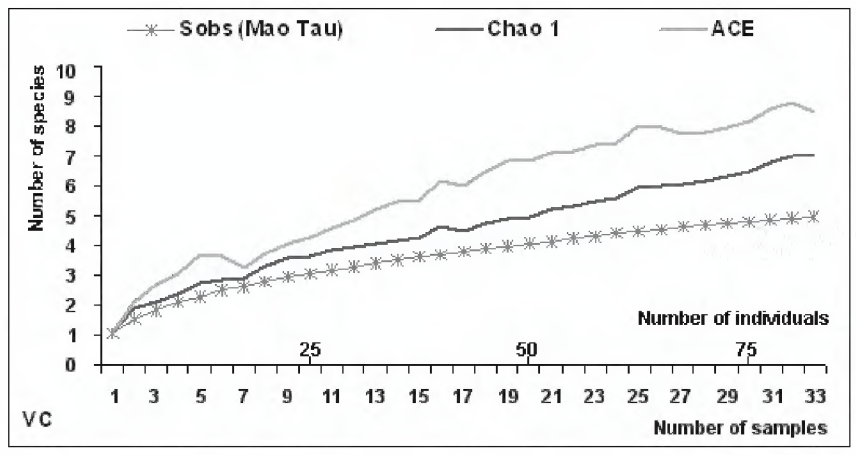
Tabel 2 - Species richness estimators computed at different spatial scales

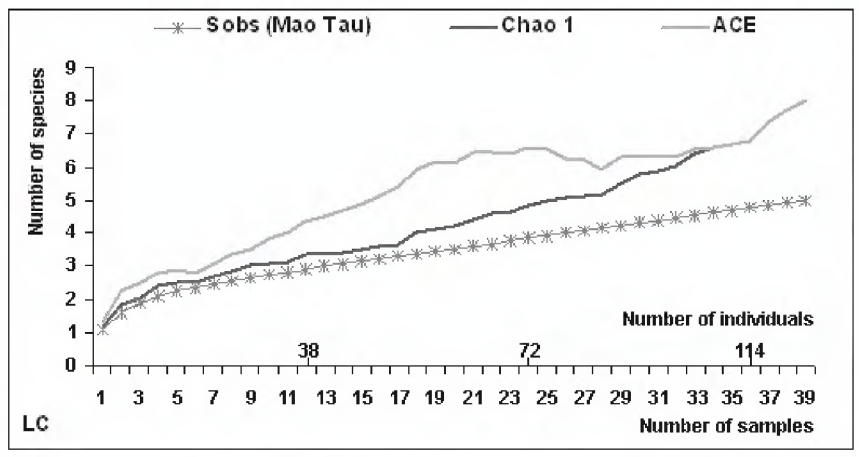
	Ungurului Cave	Vadu Crişului Cave	Peştera cu apă din Valea Leşului Cave	Ciur Izbuc Cave	Doboş Cave	Dripping water	Pools	Crişul Repede basin	Crişul Negru basin	Pădurea Craiului Mountains
No. of samples	6	33	39	47	9	67	22	78	52	17
Observed species	6	5	5	5	3	9	9	10	5	17
Chao 1 (no. of singletons/doubletons)	8 (2/1)	7 (2/2)	8 (3/0)	5 (0/0)	3 (0/0)	12 (3/0)	9 (1/0)	12 (3/2)	5 (0/0)	
ACE	8	9	8	5	3	12	9	13	5	
Chao 2 (no. of uniques/duplicates)										64 (19/5)
ICE										59

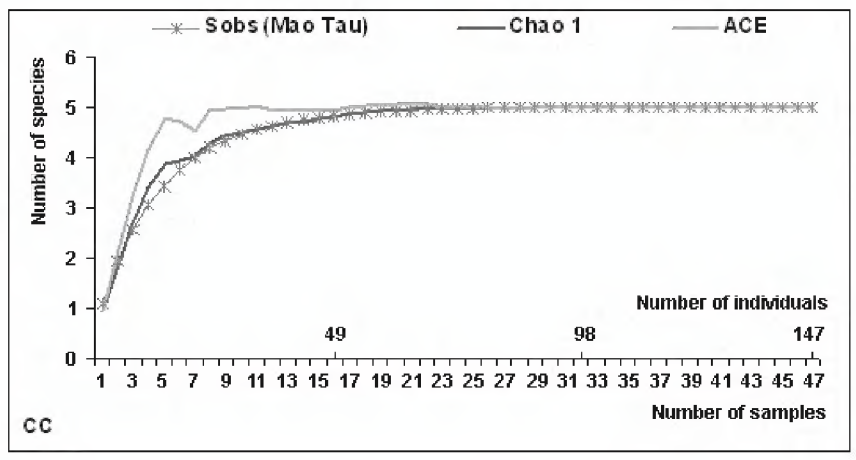
The effect of collecting from different habitats of the vadose zone on sampling completeness and species richness was tested in the five caves (UC, VC, LC, CC and DC) over seven months sampling period. The asymptote was not reached, in none of the two habitats. Nevertheless, the SAC based on drip water samples approached an asymptote, reaching 70% of the maximum number of species after 46 samples out of 67. In each of the two habitats (void network and pools), the species richness estimators failed to reach a stable value. When cumulating samples from both habitats, no differences were observed: the SACs did not reach an asymptote and the estimators failed to reach stable values (Fig. 5 a, b, c; Table 2).

Even if the sampling completeness was not achieved, the SAC based on epigean (Fig. 6 a) and on hypogean species (Fig. 6 b) approached an asymptote, reaching about 90% of the maximum observed species after 10 caves out of 11 for epigean species and after 15 caves out of 16 for hypogean species. Both species richness estimators failed to reach a stable value for hypogean species, while ICE reached the stable value of 35 epigean species after seven out of nine caves. Both estimators recorded higher estimates for patchy distribution and exhibited









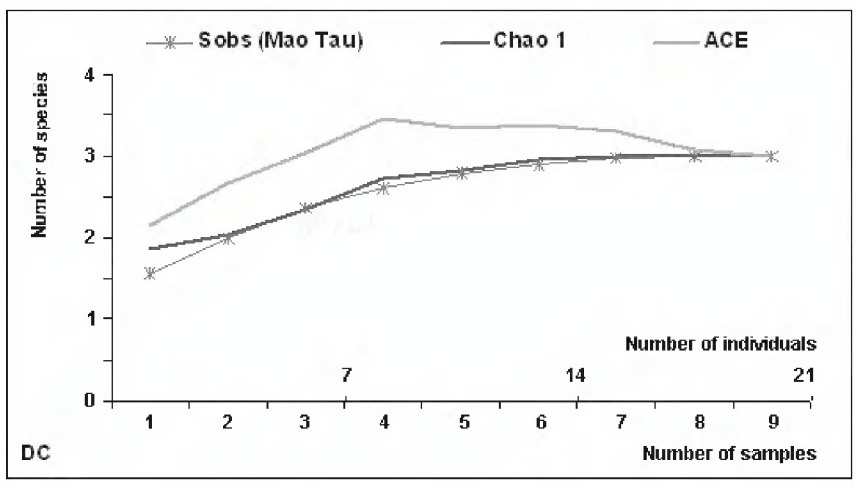
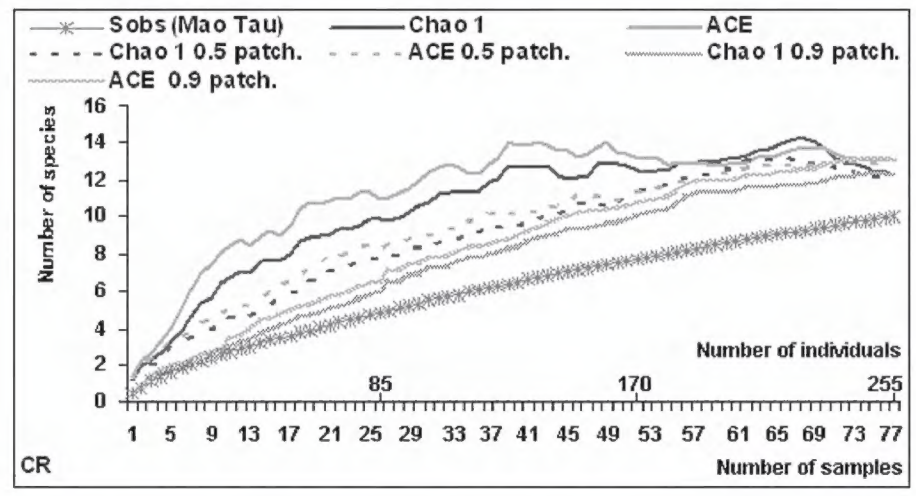


Figure 2 - Rarefaction curves of observed copepod species richness and estimates for the five studied caves: UC (6 species, 13 samples); VC (5 species, 33 samples); LC (5 species, 39 samples); CC (5 species, 47 samples); DC (3 species, 9 samples).

stronger dependence on sample size with increasing degree of patchiness, but were more stable when the analysis was computed for hypogean species.



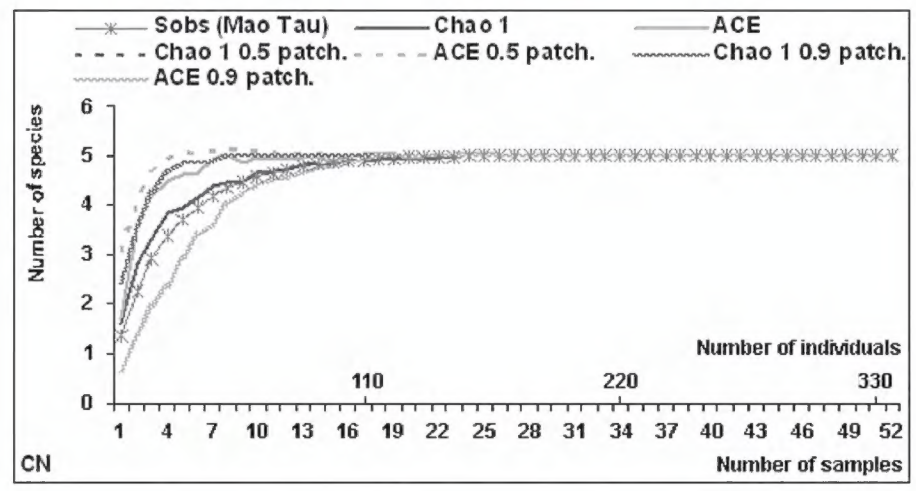


Figure 3 - Rarefaction curves of observed copepod species richness and estimates for: CR basin (10 species, 78 samples); CN basin (5 species, 52 samples) based on the cumulated data from all five caves.

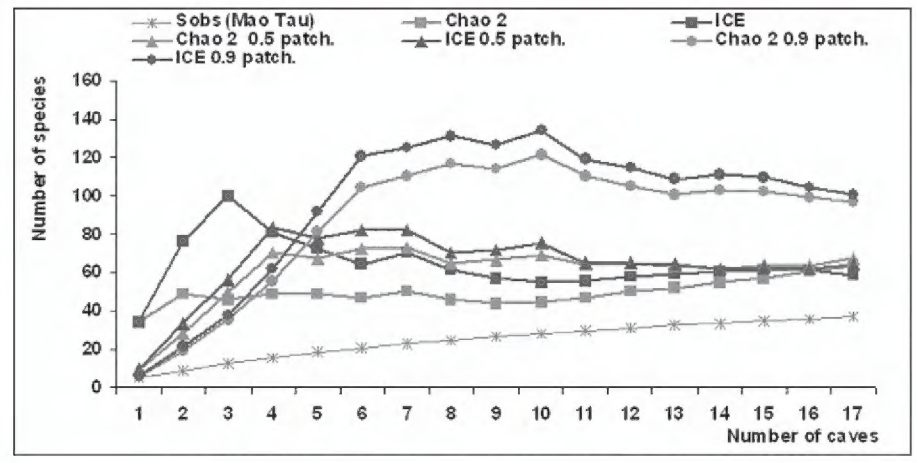
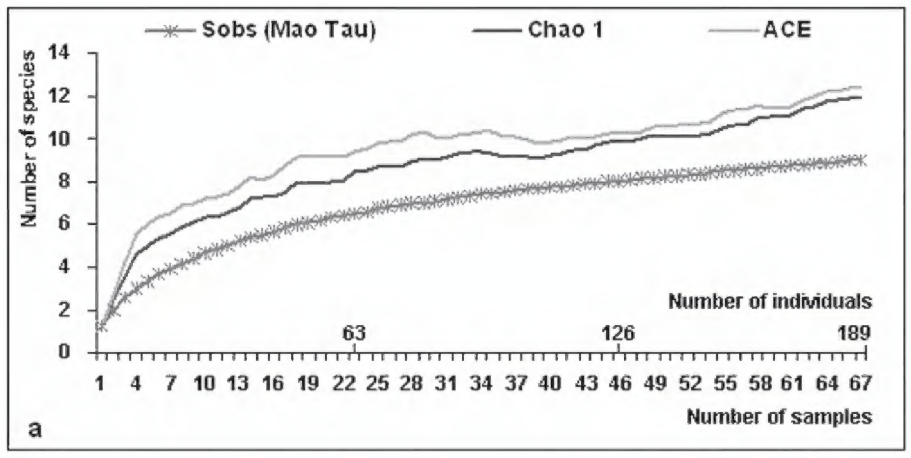


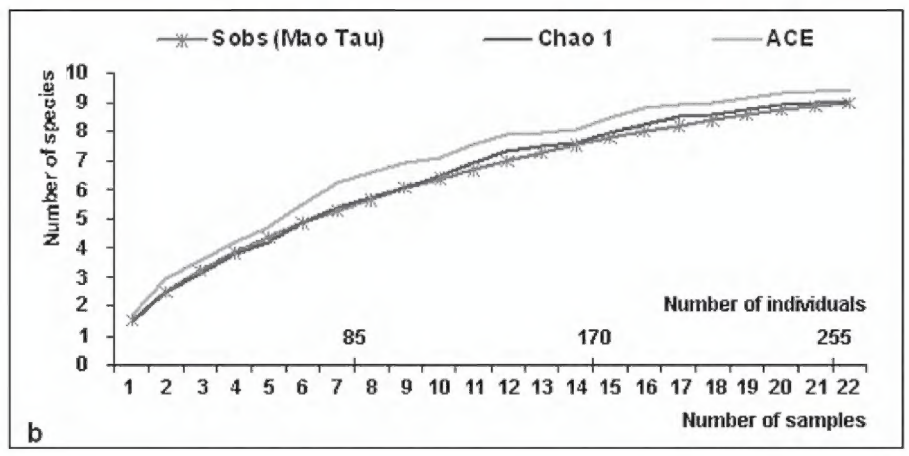
Figure 4 - Rarefaction curves of observed copepod species richness and estimates for Pădurea Craiului Mountains based on the cumulated data from all five caves (UC, VC, LC, CC, DC) and from the database (37 species, 17 caves).

DISCUSSION

From the available estimators (Chazdon et al 1998; Walther and Morand 1998; Chiarucci et al 2001; Brose 2002; Brose and Martinez 2004; Martínez-Sanz et al 2010) some were tested for a more accurate picture of species richness patterns. To date, for vadose assemblages, only rarefaction curves and Chao estimator were used to assess copepod species richness at local and regional scale in the Dinaric Mountains of Slovenia (Pipan and Culver 2007).

In our study, the selected non-parametric estimators (Chao 1, Chao 2, ACE and ICE) performed better than the accumulation curves, because usually they are less biased and more accurate than these latter ones, as al-





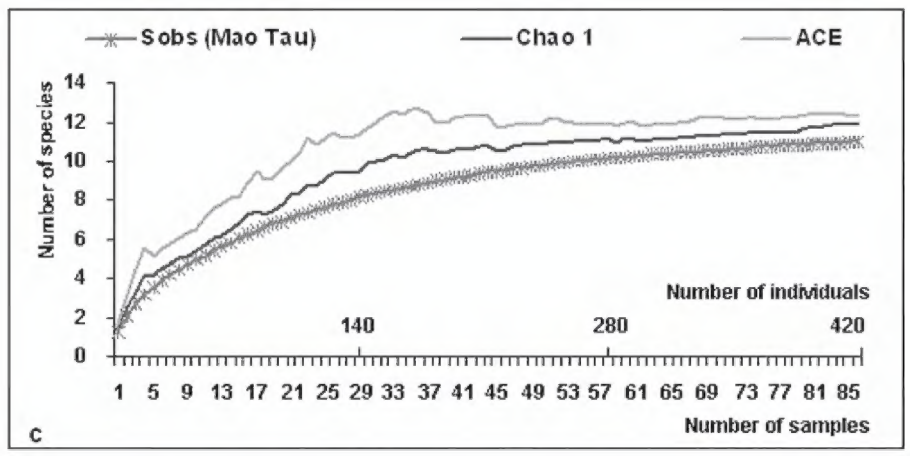
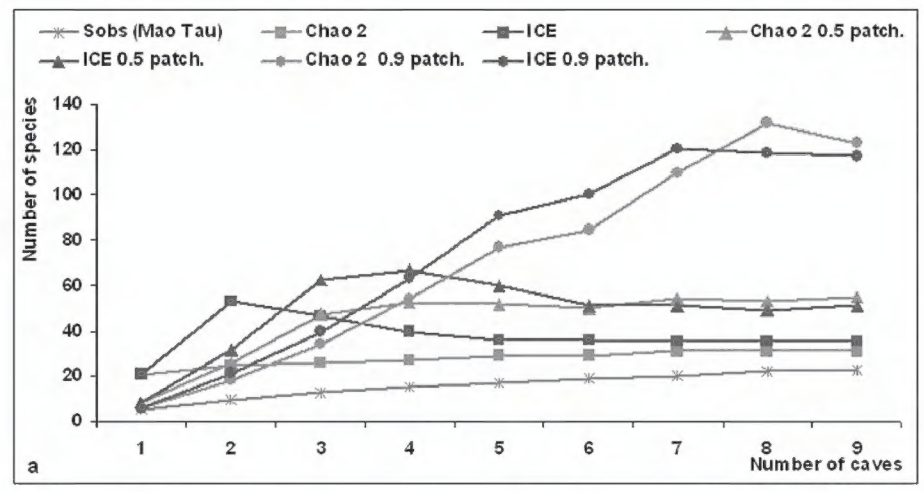


Figure 5 - Comparison of observed and estimated copepod species richness in different habitats for five caves (UC, VC, LC, CC, DC): a. dripping water (9 species, 67 samples); b. associated pools (9 species, 22 samples); c. dripping water and associated pools (11 species, 87 samples).



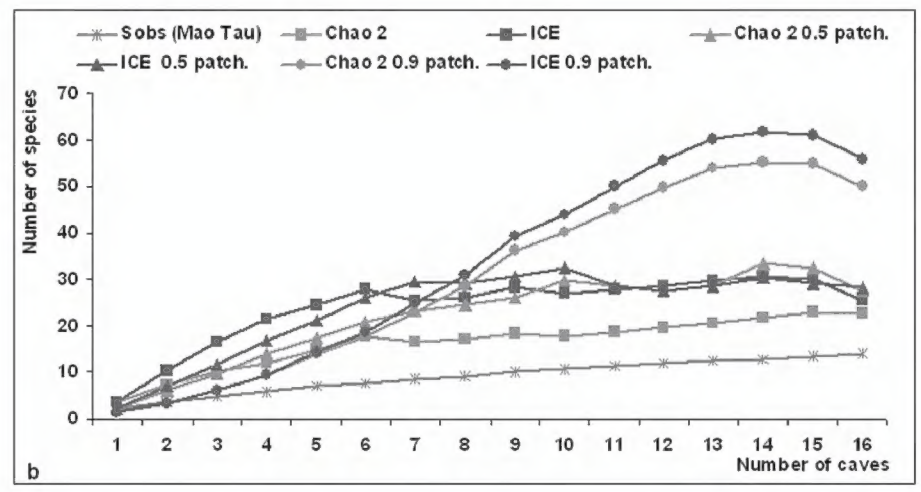


Figure 6 - Comparison of estimates of copepod species richness based on the cumulated data from five caves (UC, VC, LC, CC, DC) and from the database: a. epigean species (23 species, 9 caves); b. hypogean species (14 species, 16 caves).

ready stated by Brose et al (2003) on a landscape model. The same observation was done by Martínez-Sanz et al (2010) on macroinvertebrate communities of mountain ponds in Spain. All four non-parametric estimators could be considered good estimators, because they do not underestimate the richness measured by sampling (observed species) (Martínez-Sanz et al 2010).

The species richness estimates and the SACs obtained at cave scale were more or less similar. Abundance-based Chao 1 and ACE non-parametric estimators performed well for CC, probably because of more varied habitats observed in this cave (wide and narrow void network based on the dripping rate, pools with bottoms on clay and on calcite), which allow the presence of more species. According to Pipan and Culver (2007), for individual caves, five drips sampled for one year appeared to be sufficient to capture most of the species. In our study, in the caves where sampling completeness was achieved, nine drips (CC) and four drips (DC) sampled over one year, were sufficient to sample most of the species, reinforcing the important role played by the local context in copepod species richness.

For other caves (UC, VC and LC), where the number of singletons was high, Chao 1 was the least biased estimator, because it takes into account rare species as bearers of data about the number of possible missing species. The higher number of singletons and doubletons suggests a higher level of copepod endemism within caves, as was already stated by Pipan and Culver (2007). The ACE performance for vadose communities is in agreement with the results emphasized in other studies: ACE performed well for species-rich assemblages as Chazdon et al (1998) observed in rain forests, and are unsuitable for communities characterized by low diversity, as Walther and Morand (1998) studied on parasite communities.

Cave-specific trends of species richness could be extrapolated at basin level. Based on SAC, CN basin seems to offer more suitable spots for collecting the fauna of the vadose zone, compared to caves from the CR basin. Evidence is also the low number of hypogean species and the high number of epigean species sampled in caves from the CR basin. For the CN caves, there is a limited exchange with the surface, proved by few epigean widespread species. This leads to the conclusion that local-scale patterns in diversity tend to be more evident in stenotopic species, as was already mentioned by Adams (2009) for land plants and aquatic animals. It is the case of some hypogean species confined to different sites in caves from the CN basin (i.e. *Acanthocyclops transylvanicus*, *Bryocamptus* sp. 3, *Parastenocaris* sp. 2).

Chazdon et al (1998) defined three features for an ideal species richness estimator: 1. independence of sample size (sampling effort); 2. lack of sensitiveness to patchiness of species distribution across the samples; 3. lack of sensitiveness to sample order. In CN, the efficiency of both species richness estimators (Chao 1 and ACE) was not influenced by patchiness or by sample size, being ideal estimators at basin scale in contrast with the results obtained for CR. A possible explanation could be the already mentioned heterogeneity of habitats in CC, located in the CN basin, supporting a diverse and more specialized fauna, with heterogeneous and patchy distribution within cave. This was stated by Colwell et al (2004) hypothesizing that space and time "patchiness" among samples is influenced by the heterogeneous character of natural assemblages. This is translated in variation of the total number of species occurrences and non-random patterns of species co-occurrence among samples.

At massif level, sampling from 17 caves was not enough for assessing species richness. In the study conducted by Pipan and Culver (2007) at regional scale, drips from six caves sampled for one year seemed to offer an almost complete picture. In the present study, the species richness estimates at massif scale were higher than those obtained at both cave and basin levels, probably because the number of endemics increased with the studied hierarchical levels (cave, hydrographic basin and massif). The findings of Malard et al (2009) stated that endemic species contribution to regional richness increased with the number of sampled karst aquifers. The narrowly distributed species are the driving factor influencing the regional species richness (Stoch and Galassi 2010). The incidence-based estimators (Chao 2 and ICE) were also insensitive to the patchy species distribution, but unlike the abundance-based estimators, they were dependent on sampling size, suggesting that species richness may be properly calculated after a longer sampling effort in more caves.

Taking into account all five caves from the two basins, sampling completeness was not achieved by sampling only dripping water or by sampling only pools, even when we analyzed the cumulated results from the two habitats. Though, because the SACs based on drips approached an asymptote and copepod diversity was higher in drips than in pools, drips appear to provide a more complete picture of species richness in the vadose zone, as already observed by Pipan and Culver (2005, 2007). The higher species richness in drips could be a consequence of a more lasting habitat in the void network compared to pools, which can be dry during summer periods.

Regarding the two ecological categories, epigean vs. hypogean species, the importance of the degree of patchiness was observed in assessing copepod species richness. Even if ICE was the best estimator, it performed differently on the two ecological categories. The stable performance at higher degree of patchiness of hypogean copepods supports the hypothesis that subterranean species have patchy distribution in groundwater habitats (Galassi et al 2009). Moreover, the vadose zone characterized by habitat patchiness (Musgrove and Banner 2004) harbors species confined to peculiar microhabitats. On the contrary, the estimators of epigean copepod species reached less stable values at high degree of patchiness, probably due to the widespread distribution and euritopic requirements of surface species.

Even if many estimators are available and some of them were tested here, the estimation of species richness seems to be a difficult task. Estimators may be functional if they can give us a fair insight into the species richness trends (Hortal et al 2006). The sampling limitations and the high proportion of rare species made species richness estimation more challenging in the vadose zone. Our results highlighted how the sampling size, the degree of patchiness and the selected spatial scale influence the performance of species richness estimators, and should be taken into account when choosing the best estimator for species richness assessment. However, for abundance-based data Chao 1 is a good option being robust to sample size. For incidence-based data Chao 2 seemed to be efficient, even if it is more sensitive to the number of samples.

Finally, assessing species richness is important for conservation purposes. Different trends in copepod species richness observed at different spatial scales emphasize the importance of scale in developing and improving the strategies for monitoring protected sites.

ACKNOWLEDGEMENTS

We thank Sanda Iepure for help with the identifications of cyclopoids and Marius Vlaicu for providing the GPS coordinates of the studied caves. We thank Viorel Lascu for cave access and help on the field work and the administrations of the Ungurului (Şuncuiuş Council) and the Vadu-Crişului (the "Ţării Crişurilor" Museum) caves. We are grateful to the anonymous reviewer that improved considerably the content of this contribution. This study was financially supported by the The National University Research Council, grant IDEI_1150/2007.

REFERENCES

Adams, J. 2009. Species richness. Patterns in the Diversity of life. New York: Springer Praxis Books, Environmental Sciences.

Brancelj, A. 2002. Microdistribution and high diversity of Copepoda (Crustacea) in a small cave in central Slovenia. Hydrobiologia 477: 59–72.

Brancelj, A. 2004. Biological sampling methods for epikarst water. Pp. 99–103 in W.K. Jones, D.C. Culver, J.S. Herman, eds. Proceedings of the Symposium on

Epikarst (Shepherdstown, West Virginia, 1–4 October 2003). Charles Town, W. Va., USA: Karst Waters Institute Special Publication 9.

Brose, U. 2002. Estimating species richness of pitfall catches by non-parametric estimators. Pedobiologia 46: 101–107.

Brose, U., N.D. Martinez. 2004. Estimating the richness of species with variable mobility. Oikos 105: 292–300.

Brose, U., N.D. Martinez, R.J. Williams. 2003. Estimating species richness: sensitivity to sample coverage and insensitivity to spatial patterns. Ecology 84: 2364–2377.

Brown, J.H., S.K.M. Ernest, J.M. Parody, J.P. Haskell. 2001. Regulation of diversity: maintenance of species richness in changing environments. Oecologia 126: 321–332.

Bunge, J., M. Fitzpatrick. 1993. Estimating the number of species: A review. Journal of the American Statistical Association 88: 364–373.

Camacho, A.I., A.G. Valdecasas, J. Rodriguez, S. Cuezva, J. Lario, S. Sanchez-Moral. 2006. Habitat constraints in epikarstic water of an Iberian Peninsula cave system. Annales de Limnologie - International Journal of Limnology 42: 127–140.

Chao, A. 2005. Species richness estimation. Pp. 7909–7916 in N. Balakrishnan, C.B. Read, B. Vidakovic, eds. Encyclopedia of Statistical Sciences. New York: Wiley.

Chao, A., R.L. Chazdon, R.K. Colwell, T.-J. Shen. 2005. A new statistical approach for assessing compositional similarity based on incidence and abundance data. Ecology Letters 8:148–159.

Chao, A., M.-C. Ma, M.C.K. Yang. 1993. Stopping rules and estimation for recapture debugging with unequal failure rates. Biometrika 80: 193–201.

Chazdon, R.L., R.K. Colwell, J.S. Denslow, M.R. Guariguata. 1998. Statistical methods for estimating species richness of woody regeneration in primary and secondary rain forests of NE Costa Rica. Pp. 285–309 in F. Dallmeier, J.A. Comiskey, eds. Forest biodiversity research, monitoring and modeling: Conceptual background and Old World case studies. Paris: Parthenon Publishing.

Chiarucci, A., S. Maccherini, V. De Dominicis. 2001. Evaluation and monitoring of the flora in a nature riserve by estimation methods. Biological Conservation 101: 305–314

Colwell, R.K. 2009. EstimateS: Statistical estimation of species richness and shared species from samples. Version 8.2. User's Guide and application published at: http://purl.oclc.org/estimates

Colwell, R.K., J.A. Coddington. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society (Series B) 345: 101–118.

Colwell, R.K., C.X. Mao, J. Chang. 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. Ecology 85: 2717–2727.

Ford, D., P. Williams 2007. Karst Hydrogeology and Geomorphology. John Wiley & Sons, Ltd.

Galassi, D.M.P., R. Huys, J. Reid. 2009. Diversity, ecology and evolution of groundwater copepods. Freshwater Biology 54: 691–708.

Gaston, K.J. 1996. Species richness: measure and measurement. Pp. 77–113 in K.J. Gaston, ed. Biodiversity. A Biology of Numbers and Difference. Oxford: Blackwell Science.

Gibert, J. 2001. Basic attributes of groundwater systems. Pp. 35–52 in Ch. Griebler, D.L. Danielopol, J. Gibert, H. Nachtnebel, J. Notenboom, eds. Groundwater ecology. A tool for management of water resources. Belgium: Office for Official Publications of the European Communities.

Hortal, J., P.A.V. Borges, C. Gaspar. 2006. Evaluating the performance of species richness estimators: sensitivity to sample grain size. Journal of Animal Ecology 75: 274–287.

Lee, S.-M., A. Chao. 1994. Estimating population size via sample coverage for closed capture-recapture models. Biometrics 50: 88–97.

Lescher-Moutoué, F. 1973. Sur la biologie et l'écologie des Copépodes Cyclopides hypogés (Crustacés). Annales de Spéléologie 28: 429–502, 581–674.

Magurran, A.E. 2004. Measuring biological diversity. Oxford: Blackwell Publishing.

Malard, F., C. Boutin, A.I. Camacho, D. Ferreira, G. Michel, B. Sket, F. Stoch. 2009. Diversity patterns of stygobiotic crustaceans across multiple spatial scales in Europe. Freshwater Biology 54: 756–776.

Mangin, A. 1994. Karst hydrogeology. Pp. 43–64 in J. Gibert, D.L. Danielopol, J. Stanford, eds. Groundwater Ecology. San Diego, California: Academic Press.

Martínez-Sanz, C., F. García-Criado, C. Fernández Aláez, M. Fernández Aláez. 2010. Assessment of richness estimation methods on macroinvertebrates communities of mountain ponds in Castilla y León (Spain). Annales de Limnologie - International Journal of Limnology 46: 101–110.

Meleg, I.N., O.T. Moldovan, S. Iepure, F. Fiers, T. Brad. 2011. Diversity patterns of fauna in dripping water of caves from Transylvania. Annales de Limnologie – International Journal of Limnology 47: 185–197.

Moldovan, O.T., T. Pipan, S. Iepure, A. Mihevc, J. Mulec. 2007. Biodiversity and Ecology of Fauna in Percolating Water in Selected Slovenian and Romanian Caves. Acta Carsologica 36: 493–501.

Musgrove, M., J.L. Banner. 2004. Controls on the spatial and temporal variability of vadose dripwater chemistry: Edwards Aquifer, central Texas. Geochimica et Cosmochimica Acta 68: 1007–1020.

Pipan, T. 2005. Epikarst – A promising habitat. Postojna – Ljubljana: ZRC Publishing, Karst Research Institute at ZRC SAZU.

Pipan, T., A. Brancelj. 2001. Ratio of copepods (Crustacea: Copepoda) in fauna of percolation water in six

karst caves in Slovenia. Acta Carsologica 30: 257–265.

Pipan, T., A. Brancelj. 2004a. Diversity and peculiarity of epikarst fauna: Case study from six caves in Slovenia (Europe). Pp. 119–126 in W.K. Jones, D.C. Culver, J.S. Herman, eds. Proceedings of the Symposium on Epikarst (Shepherdstown, West Virginia, 1–4 October 2003). Charles Town, W. Va., USA: Karst Waters Institute Special Publication 9.

Pipan, T., A. Brancelj. 2004b. Distribution patterns of copepods (Crustacea: Copepoda) in percolation waters of the Postojnska Jama Cave System (Slovenia). Zoological Studies 43: 206–210.

Pipan, T., D.C. Culver. 2005. Estimating biodiversity in the epikarstic zone of a West Virginia cave. Journal of Cave and Karst Studies 67 (2): 103–109.

Pipan, T., D.C. Culver. 2007. Epikarst communities: biodiversity hotspots and potential water tracers. Environmental Geology 53: 265–269.

Rouch, R. 1968. Contribution à la connaissance des Harpacticides hypogés (Crustacés – Copépodes). Annales de Spéléologie 23: 5–167.

Rouch, R. 1982. Le système karstique du Baget. XII. La communauté des harpacticides. Sur l'interdépendance des nomocénoses épigée et hypogée. Annales de Limnologie 18 (1): 41–54.

Rouch, R., A. Carlier. 1985. Le système karstique du Baget. XIV. La communauté des harpacticides. Evolution et comparaison des structures du peuplement epigé a l'entrée et a la sortie de l'aquifère. Stygologia 1 (1): 71–92.

Rusu, T. 1988. Pe urmele apelor subterane. Carstul din Munţii Pădurea Craiului. Cluj – Napoca: Ed. Dacia.

Sket, B., P. Trontelj, C. Žagar. 2004. Speleobiological characterization of the epikarst and its hydrological neighborhood: its role in dispersion of biota, its ecology and vulnerability. Pp. 104–113 in W.K. Jones, D.C. Culver, J.S. Herman, eds. Proceedings of the Symposium on Epikarst (Shepherdstown, West Virginia, 1–4 October 2003). Charles Town, W. Va., USA: Karst Waters Institute Special Publication 9.

Stoch, F. 1997. A new genus and two new species of Canthocamptidae (Copepoda, Harpacticoida) from caves in northern Italy. Hydrobiologia 350: 49–61.

Stoch, F. 2000. New and little known *Parastenocaris* (Copepoda, Harpacticoida, Parastenocarididae) from cave waters in Northeastern Italy. Bollettino del Museo Civico di Storia Naturale di Verona 24: 195–206.

Stoch, F., D.M.P Galassi. 2010. Stygobiotic crustacean species richness: a question of numbers, a matter of scale. Hydrobiologia 653: 217–234.

Walther, B.A., S. Morand. 1998. Comparative performance of species richness estimation methods. Parasitology 116: 395–405.